REMARKS

The Office Action mailed April 24, 2006 has been carefully considered and the following is responsive thereto.

Claims 3-12 and 15-21 are pending in the application. Claims 10 and 16 have been amended and claims 12 and 15 have been canceled without prejudice. Claims 11 and 17 have been amended to correct typographical errors. New claim 22 directed to the method of claim 10 wherein the postoperative complications are ischemia-reperfusion injury has been added. No new matter has been added.

At page 2 of the Office Action the Examiner rejected claims 3-12 and 15-21 under 35 USC 112, second paragraph as being indefinite. The Examiner alleged that claims 10, 12 and 16 are rendered vague and indefinite by the phrase "gastrointestinally administering a composition" (claim 10) and the phrase "for gastrointestinal administration" (claim 16) because it is unclear to whom or what the composition is being administered. Further, the Examiner alleged that claims 10 and 15 are rendered vague and indefinite by the phrase "for the support of surgical patients" because it is unclear what is meant by the term "support".

Applicants traverse this rejection. Claim 10 has been amended to delete the term "support" and add that the composition is administered to a surgical patient. Claims 12 and 15 have been canceled without prejudice. Claim 16 has been amended to state that the composition is for gastrointestinal administration to a surgical patient. In view of the above, withdrawal of this section 112, second paragraph rejection is respectfully requested.

At pages 2-3 of the Office Action, the Examiner rejected claims 3-12 and 15-21 under 35 USC 103 as being unpatentable over Inanami et al. (Free Radic Res), Schneider et al. (U.S. Patent 6,656,608) and Jerkic et al. (Nephr Dial Trans) and further in view of Wu et al. (J. Nutr.).

Applicants traverse this rejection. Applicants respectfully submit that a *prima facie* case of obviousness has not been established with regard to claims 3-11 and 16-21.

A *prima facie* case of obviousness requires the following: (1) there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings; (2) there must be a reasonable expectation of success; and (3) the prior art reference (or references when combined) must teach or suggest all the claim limitations. MPEP at 2143.

Inanami et al. discloses oral administration of (-)catechin from green tea to gerbils for two weeks prior to surgery to induce transient focal brain ischemia, and continuing one week after surgery. The authors found that oral administration of (-) catechin protected the animals against ischemia-reperfusion-induced neuronal death.

Schneider et al. (U.S. Patent 6,656,608) disclose the use of one or more of the amino acids glycine, alanine and serine in combination with a) omega-3 polyunsaturated fatty acids; b) arginine or ornithine or pharmaceutically acceptable salt of arginine or ornithine; or c) RNA, nucleotide or nucleoside; or mixtures of one or more of a), b) and c) to prevent or minimize the effects of hypoxia-reperfusion injury. When used to minimize the effects of ischemia-reperfusion injury, column 7, lines 9-13 disclose that a dietary supplement containing the foregoing can be administered over a period of three days or longer before surgery, generally three to six days before surgery. Such supplements are disclosed at column 6, lines 21-61 as comprised of energy sources in an amount supplying from 600 to 1,000 Kcal/day. Schneider et al. does not disclose or suggest administration of green tea extract for any purpose, much less to prevent or reduce postoperative complications.

Jerkic et al. discloses administration of arginine, a NO substrate, to rats for four weeks prior to induction of acute renal failure. The authors found that arginine reduces tubular cell injury in acute post-ischemic renal failure. Jerkic et al. does not disclose or suggest administration of green tea extract for any purpose, much less to prevent or reduce postoperative complications.

Wu et al. provides a review of studies concerning the role of arginine on cardiovascular function and therapy. At page 2628, right column discloses that studies using animal models

suggest that arginine administration improves tissue preservation during reperfusion and increases regional blood flow in focal cerebral ischemia.

None of the cited references, alone or in any combination, disclose or suggest the method of amended claim 10 of averting or reducing the risk of postoperative complications wherein a composition comprising a) green tea extract and b) at least one NO donor which is a substrate of NO synthetase, and/or one precursor of this NO donor is gastrointestinally administered to a surgical patient, wherein administration of the composition takes place less than twenty-four hours before a surgical procedure.

The Examiner alleged that Inanami et al. teaches the use of green tea extract. Inanami et al. does not suggest or disclose the use of green tea extract to protect against ischemia-reperfusion injury, nor is there any suggestion or disclosure that tea extract can be substituted for (-)catechin. As disclosed in the specification at page 8, lines 20-29, green tea contains a number of constituents from different classes of compounds, including amino acids, polyphenols, vitamins, saccharides, minerals and caffeine. Rao et al. "Pharmacological functions of green tea polyphenols", *In* Performance functional foods (D.H. Watson, ed.), Woodhead publications Ltd, CRC Press, pages 140-159, 2003, a copy of which is submitted herewith as Exhibit A, shows the composition of green tea in Table 9.1. Green tea extract contains many compounds other than (-)catechin. Green tea extract cannot be considered the same as or the equivalent of (-)catechin. Persons skilled in the art know that from the properties of a single component which is optionally present in green tea extract, it cannot be concluded that the properties of a mixture containing this component will show the same or similar properties. As green tea extract possesses different ingredients of different chemical classes with different functions it is certain that the properties and effects of green tea extract do not match the exact properties and effects of catechins.

Applicants have surprisingly found that administration of the claimed composition to a surgical patient less than 24 hours before a surgical procedure averts or reduces the risk of postoperative complications. There is nothing in any of the cited references, alone or in any combination that suggests the claimed composition and a method of averting or reducing the

risk of postoperative complications by gastrointestinally administering to a surgical patient a composition comprising a) green tea extract and b) at least one NO donor which is a substrate of NO synthetase, and/or one precursor of this NO donor before a surgical procedure wherein administration of the composition takes place less than twenty-four hours before a surgical procedure.

Inanami et al. discloses oral administration of (-)catechin from green tea to gerbils for two weeks prior to surgery. Schneider et al. discloses that when used to prevent or minimize the effects of hypoxia-reperfusion injury a dietary supplement can be administered over a period of three days or longer before surgery, generally three to six days before surgery. Jerkic et al. discloses administration of arginine, a NO substrate, to rats for four weeks prior to induction of acute renal failure. Wu et al. discloses that studies using animal models suggest arginine administration improves tissue preservation during reperfusion and increases regional blood flow in focal cerebral ischemia, but does not disclose when the arginine was administered. There is nothing in any of the cited references alone or in any combination that suggests the much shorter time period of less than 24 hours before a surgical procedure as recited in the method of claim 10.

Claims 3-11 and 16-22 are not *prima facie* obvious in view of Inanami et al., Schneider et al. and Jerkic et al. in view of Wu et al. (J. Nutr.). None of the prior art references, alone or in any combination, teach or suggest all of the limitations of claims 3-11 and 16-22. Withdrawal of this section 103 rejection is respectfully requested.

In view of the above, the present application is believed to be in a condition ready for allowance. Reconsideration of the application is respectfully requested and an early Notice of Allowance is earnestly solicited.

The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 03-2775, under Order No. 09600-00031-US. A duplicate copy of this paper is enclosed.

Date: September 25, 2006

Respectfully submitted,

By Liza D. Hohenschutz

Liza D. Hohenschutz
Registration No.: 33,712
CONNOLLY BOVE LODGE & HUTZ LLP
1007 North Orange Street
P.O. Box 2207
Wilmington, Delaware 19899
(302) 658-9141
(302) 658-5614 (Fax)
Attorney for Applicant

488650

Exhibit A

9

Pharmacological functions of green tea polyphenols

T. P. Rao, T. Okubo, D-C. Chu and L. R. Juneja, Taiyo Kagaku Co. Ltd, Japan

9.1 Introduction

Green tea is a simple refreshing beverage consumed by millions of people around the world, and its medicinal properties have been recognized for many centuries. It was even referred to in Chinese literature written in 200 BC as the remedy for various illnesses (Hu, 1986). However, until recently its medicinal properties have not been appreciated in the modern world. Currently green tea is provoking great interest within the medical community because of mounting scientific evidence on its powerful antioxidant activity in preventing various diseases.

The scientific evidence suggests that this simple beverage contains large quantities of low molecular weight polyphenols (Table 9.1), that are found to be more potent antioxidants and free-radical scavengers than are vitamin C or vitamin E (Rice-Evans et al., 1996). The green tea polyphenols are mainly composed of six kinds of catechins (also known as tannins), epigallocatechin gallate (EGCg), epigallocatechin (EGC), epicatechin gallate (ECg), epicatechin (EC), gallocatechin (GC) and catechin (C). Among the six, EGCg is the main component available in large quantities in either crude or refined green tea extracts (Table 9.2). The chemical structures of these polyphenols indicate that they belong to a class of flavan-3-ol, which is composed of C_{15} (fifteen carbon atoms) compounds; their derivatives are composed of two phenolic nuclei (A and B rings) connected by three carbon units (2, 3 and 4) of C ring (Fig. 9.1). The significance of these compounds in medical use is their ability to react with various substances. The reactions of polyphenols with substances such as soybean lipoxygenase (Sekiya et al., 1984), caffeine (Martin et al., 1986; Murayama et al., 1991),

Table 9.1 Composition of green tea (% of dry weight)

Components	%
Proteins	24.0
Carbohydrates	45.8
Lipids	4.6
Polyphenols	13.0
Caffeine	2.3
Ash	5.4
Vitamins A and B	0.02
Vitamin C	0.25

Anonymous (1996), *Standard tables of food composition in Japan*, Tokyo, Resource Council of Science and Technology, 198–9

Table 9.2 Composition of polyphenols in crude (hot water infusion) and refined (Sunphenon® 100S) green tea extract

Name of the catechin		% of dry extract	
		Crude	Refined
(−) – Epigallocatechin gallate	(EGCg)	10.0	31.5
(−) – Epigallocatechin	(EGC)	8.9	17.3
(+) – Gallocatechin	(GC)	3.3	9.4
(−) – Epicatechin	(EC)	4.1	5.9
(−) – Epicatechin gallate	(ECg)	3.9	5.1
(−) – Catechin	(C)	1.9	2.8

cystine (Richard et al., 1991) and methylmercaptan (Yasuda and Arakawa, 1995) indicated that their reactions mainly involve conjugation at the 3′ and 4′ positions on the B ring. It has been suggested that green tea polyphenols act as a powerful radical scavenger through these chemical reactions in preventing various diseases.

In the last two decades, various *in vitro* and *in vivo* studies have indicated the antimicrobial and antioxidant properties of green tea polyphenols. Recent clinical studies have confirmed their antioxidant properties in preventing various ailments such as cardiovascular diseases, cancer, renal failure and allergy. In addition to this they were found to be powerful in the suppression of growth and enzyme synthesis of various pathogenic bacteria and viruses that are harmful to humans. In this chapter, the pharmacological functions of green tea polyphenols are classified into antibacterial, antiviral and antioxidant activities. We also discuss their functions in relation to the above disorders with an emphasis on clinical results.

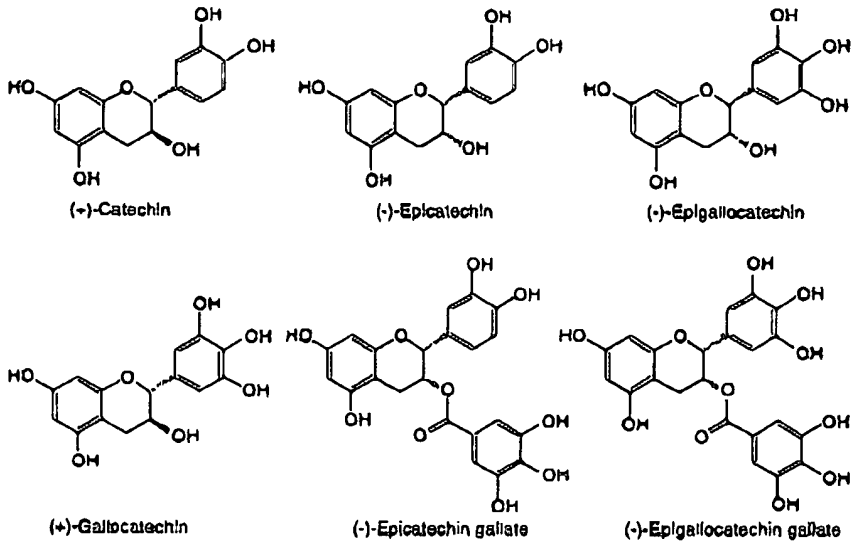

Fig. 9.1 The structures of green tea polyphenols.

9.2 Antibacterial activity

Green tea polyphenols have very strong antibacterial activity against various pathogens (Juneja et al., 2000). In this section, antibacterial activities against oral bacteria, intestinal bacteria and foodborne bacteria that are relevant to human health are discussed.

9.2.1 Oral care

The benefits of green tea consumption on oral care were first noticed in the middle of the 1970s in Japan, when a reduction in the rate of dental caries with the consumption of green tea was observed in school children (Onisi et al., 1981a; Onisi 1985). Similar studies in China also linked the consumption of green tea with lowered incidence of tooth decay (Jin et al., 1991). The effect of green tea in the prevention of dental caries was once thought to be due to fluorides contained in the tea leaves. Later it had been noticed that the tea infusion was more effective in decreasing dental caries than the application of the fluoride solution itself (Onisi et al., 1981b; Yu et al., 1992). Further studies *in vitro* and *in vivo* pointed out that polyphenols present in green tea are the real functional compounds in the suppression of tooth decay. Detailed studies suggested that the antibacterial activity of green tea polyphenols against cariogenic bacteria is the basis for the prevention of tooth decay (Muroi and Kubo, 1993). Green tea polyphenols

Table 9.3 Minimum inhibitory concentrations (MIC: $\mu g\,ml^{-1}$) of green tea polyphenols on several pathogenic oral bacteria. The inhibition of the bacterial growth was observed in sensitive meat extract agar medium

Bacterial species and strains	Green tea polyphenols					
	C	EC	GC	EGC	ECg	EGCg
Streptococcus mutans						
MT8148	>1000	>1000	250	250	1000	500
IFO 13955	>1000	>1000	250	250	>1000	500
Streptococcus sobrinus						
6715DP	>1000	>1000	250	250	>1000	500
Porphyromonas gingivalis						
381	1000	1000	1000	1000	1000	500
ATCC 33277	1000	1000	1000	1000	1000	250
GAI	1000	1000	1000	1000	1000	500

Sakanaka et al., 1997

were also recognized as a powerful deodorant in the alleviation of bad oral odor and therefore improved oral health (Chu and Juneja, 1998).

Prevention of tooth decay

The main causes of tooth decay are dental caries and periodontal diseases that are induced by oral microflora. Among the several hundreds of microorganisms indigenous to oral cavities, *Streptococcus mutans* is predominantly cariogenic (Hamada and Slade, 1980; Loesche, 1986). The growth and cariogenicity of this bacterium depend on the availability of sucrose from food. It synthesizes water-insoluble glucans (plaque formation) catalyzed by glucosyltransferase (GTase) in the presence of sucrose and in this way adheres strongly to the tooth surface causing tooth decay (Hamada and Slade, 1980). *In vitro* studies on the green tea polyphenols were found to inhibit the growth of this bacterium, glucan synthesis and the cellular adherence of cariogenic *S. mutans* (Hattori et al., 1990; Otake et al., 1991; Sakanaka et al., 1989, 1990, 1992). The minimum inhibitory concentrations (MIC) of green tea polyphenols on the growth of *Streptococcus* species were in the range of 250 to $1000\,\mu g\,ml^{-1}$ (Table 9.3). In another study, Sakanaka et al. (1990) reported that glucan synthesis by the above bacteria was strongly inhibited by ECg and EGCg of green tea extract. They also found that even as little as $25–30\,\mu g\,ml^{-1}$ of ECg and EGCg could completely inhibit glucan synthesis and a concentration of $50\,\mu g\,ml^{-1}$ of both components could completely inhibit adherence of the bacterial cells.

Oiwa et al. (1993) and Terajima et al. (1997) conducted clinical studies with human volunteers to examine the effect of Sunphenon® (a commercial product of green tea polyphenols, Taiyo Kagaku Co., Ltd., Japan) on plaque formation. In these studies, the volunteers were asked to rinse their

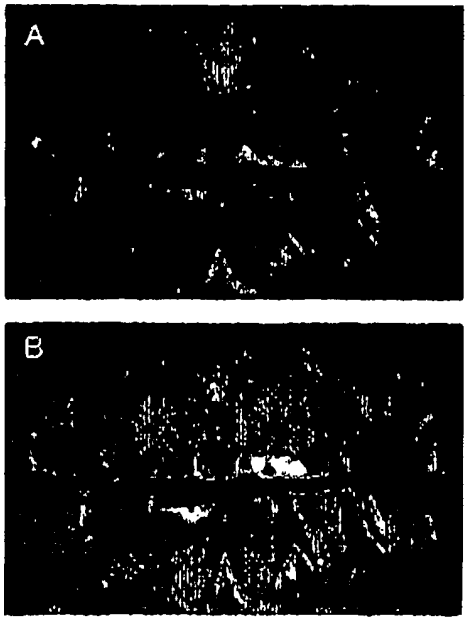

Fig. 9.2 Effect of green tea polyphenols on dental caries plaque formation. A, without and B, with the application of green tea polyphenols as mouth wash.

teeth either with water or with a solution containing 0.05–0.5% of green tea polyphenols for 20 seconds. They did this three times a day after meals for three consecutive days. They were forbidden to brush their teeth during the test period. After these treatments, dental plaque formation was identified by staining with prospec dye and photographed. In a visual comparison, a significant inhibition of plaque formation was noticed in those volunteers administered with green tea polyphenol (Fig. 9.2). In a continuation of the study, the same volunteers were divided into four groups and administered with 0.05, 0.1, 0.2 and 0.5% green tea polyphenols for another three days in the same procedure. Photographs were taken to calculate the inhibition rate of plaque formation. As shown in Table 9.4, dental plaque formation decreased in the volunteers who rinsed their teeth with green tea polyphenols. The inhibition rate of plaque formation was 30–43% in the test groups (Sakanaka et al., 1997) and so the green tea polyphenols are recognized as an effective inhibitor of plaque formation in humans.

Green tea polyphenols are also effective in the inhibition of growth and adherence of another bacterium, *Porphyromonas gingivalis*, which causes periodontal disease (Kakuda et al., 1994; Sakanaka et al., 1996). The adherence of *P. gingivalis* to oral epithelial cells is the initial step in the pathogenesis of periodontitis. *In vitro* experiments showed that Sunphenon® at

Table 9.4 Effect of green tea polyphenols on dental plaque formation in men

Polyphenols in mouth wash solution (%)	Inhibition rate (%)
0.05	41.0
0.1	33.9
0.2	30.6
0.5	43.1

Sakanaka et al., 1997

concentrations of 0.1 mg ml^{-1} and above strongly inhibited the adherence of *P. gingivalis* to epithelial cells (Sakanaka et al., 1996). EGCg, the active component of green tea polyphenols, suppressed adherence of the bacterium onto human buccal epithelial cells at a concentration of 250–500 μg ml^{-1} (Table 9.3). Clinical tests have revealed a substantial decrease in levels of this bacterium with the application of green tea in periodontitis patients (Nawashiro et al., 1996).

Collagenase, which is produced by *P. gingivalis*, breaks down collagen in the gums, weakens the periodontal pocket and eventually recedes the gums leading to gingival and periodontal diseases. The application of Sunphenon® almost completely inhibited the activity of collagenase at concentration of 50 μg ml^{-1}, and the enzyme activity was completely inhibited at 100 μg ml^{-1} (Juneja et al., 2000).

One may expect that the effectiveness of the polyphenols as anticariogenic agents may depend on the duration of their availability at minimum inhibitory concentrations in saliva. The minimum inhibitory concentrations of catechins to cariogenic *Streptococci* and other related bacteria are 250–500 μg ml^{-1} or less (Sakanaka et al., 1989). Among the catechins, EGCg, ECg and GCg are effective inhibitors of both *S. mutans* and *P. gingivitis* (Sakanaka et al., 1990, 1996). These components have shown the enzyme-inhibitory effect even at 25–30 μg ml^{-1} or less (Sakanaka et al., 1990). Tsuchiya et al. (1997) analyzed the constituents of human saliva after the intake of green tea extract at a concentration of 5 mg ml^{-1}. They found that catechins at such concentrations were retained in saliva for up to 60 min after the intake of green tea extract (Table 9.5). These results suggest that green tea polyphenols can be used as effective natural anticariogenic agents.

Prevention of halitosis

In modern life, diversified food and drink intake induces several kinds of oral odor or halitosis (Chu and Juneja, 1998). The odor is mainly caused by the proteins of epithelial organization, connective tissues, food residues and

Table 9.5 Green tea polyphenols concentrations ($\mu g\,ml^{-1}$) in saliva after mouthwash with green tea solution ($5.0\,mg\,ml^{-1}$)

Time after mouth wash (min)	Green tea polyphenols concentrations ($\mu g\,ml^{-1}$)					
	C	EC	GC	EGC	ECg	EGCg
1	11.9	20.5	26.7	38.2	43.4	165.1
10	3.41	7.42	9.58	17.6	15.4	52.1
30	1.75	4.34	6.41	10.2	7.57	24.7
60	1.65	2.8	5.39	7.02	5.24	16.1

Tsuchiya et al., 1997

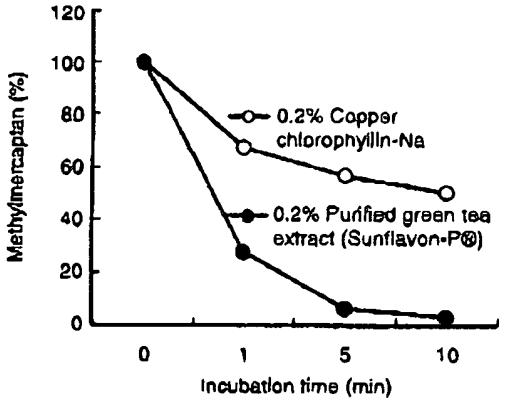

Fig. 9.3 Effect of purified green tea extract (Sunflavon P®) on the inhibition of oral odor in comparison with copper chlorophyllin-Na, a normal deodorant.

bacteria in the mouth. The proteins are dissolved by enzymes to produce odorous volatile sulfide substances. Among several odorous sulfide gases, methylmercaptan (CH_3SH) has a strong relationship with oral odor (Ui et al., 1991).

The components of green tea polyphenols were reported to have strong deodorant activity against CH_3SH (Ui et al., 1991). The purified green tea extract (Sunflavon-P®) showed stronger deodorant activity in the suppression of oral CH_3SH content than copper chlorophyllin-Na, a common deodorant (Fig. 9.3). Other natural substances from different plant extracts also possess deodorizing activity; most of them are polyphenols and phenolic derivates (Tokita et al., 1984; Yasuda and Ui, 1992). Among the six tea catechins, EGCg had the strongest deodorizing activity against CH_3SH (Ui et al., 1991). The deodorizing mechanism of polyphenols against CH_3SH is

considered to involve the hydrogen bonds between phenolic hydroxyl groups and the thiol group (Yasuda and Arakawa, 1995). The order of deodorizing activity of green tea polyphenols was EGCg > EGC > ECg > GA > EC (Ui et al., 1991; Yasuda and Arakawa, 1995), which is similar to the order of molar intensity for antioxidant activity of these catechins (Matsuzaki and Hara, 1985; Rice-Evans et al., 1996). This suggests a link between deodorant and antioxidant activities of these catechins.

9.2.2 Harmonizing enteric microflora

Hundreds of microbial species, millions in cell counts, are native to the intestine. These microbes are involved in various physiological functions, of which the majority are beneficial to the human body, but some are very harmful (Okubo and Juneja, 1997). Most of the species constitute lactic acid bacteria, such as certain *Bifidobacterium* and *Lactobacillus* genera that play a significant role in metabolism (digestion), host-defense against infection, ageing and immunopotentiation (Hentges, 1983; Mitsuoka, 1984) and so these species of bacteria are generally considered beneficial. On the other hand, some harmful bacteria belonging to certain clostridial species, *Clostridium perfringens* and *C. difficile* are found and these are closely linked to intestinal diseases and tumor growth (Bokkenheuser, 1983; Gary and Sherwood, 1984; Goldman, 1983). It is therefore necessary to maintain a balance among these microflora.

The composition of gut microflora is largely affected by diet and by age (McCarthy and Salyers, 1988; Rowland et al., 1985; Salyers and Leedle, 1983). A healthy diet with sufficient oligosaccharides and dietary fiber was found to promote useful bacteria and suppress the harmful ones. Similarly, green tea polyphenols with antibacterial activity exhibited a favorable influence on the composition of the intestinal microflora.

In vitro studies on the green tea polyphenols have shown a selective, growth inhibitory activity against various harmful *Clostridia* species, while having little effect on other bacteria (Ahn et al., 1990a, b). All the catechins except EGC of green tea polyphenols have some growth inhibitory effect on *C. perfringens* (Table 9.6). However, EGCg and ECg have the highest degree of inhibitory effect on both *C. difficile* and *C. perfringens*. (Ahn et al., 1991). These results suggest a certain relationship between the structures of polyphenols and the growth inhibitory effect. The gallate moiety linked by an ester linkage in the polyphenol molecules seems to be related to bacterial growth inhibition activity.

Okubo et al. (1992) conducted a clinical study to investigate the effect of green tea polyphenols (in the form of Sunphenon®) on the growth of the human intestinal microflora. Eighty healthy volunteers were administered with 0.4 g polyphenols after each meal (i.e., 3 times a day) for 8 weeks in the following schedules. In the first two weeks (weeks 1 and 2) the volunteers did not take the polyphenols, the following two weeks (weeks 3

Table 9.6 Growth inhibitory activity of tea polyphenols against *C. difficile* ATCC-9689 and *C. perfringens* ATCC-13124

Polyphenols	*C. difficile*	*C. perfringens*
C	*	+
EC	–	+
GC	–	+
ECg	++	++
ECG	–	–
EGCg	++	++

*, no inhibition; +, inhibition and ++, strongest inhibition
Ahn et al., 1991

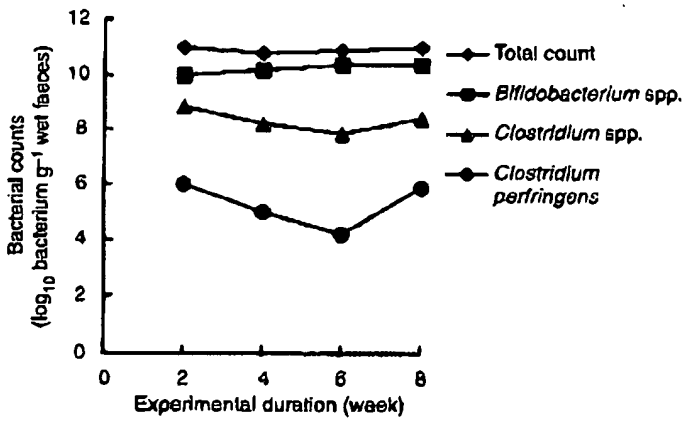

Fig. 9.4 The changes in the intestinal microflora during administration (2 to 6 weeks) and post administration (6 to 8 weeks) of green tea polyphenols in human volunteers.

and 4) and the next two weeks (weeks 5 and 6) they took the polyphenols and the last two weeks (weeks 7 and 8) they again did not take the polyphenols. The composition of different species of bacteria in the faeces was examined at the end of each test period (after every two weeks). The results were inconsistent with those of *in vitro* studies. The data indicated a decrease in the bacterial populations of *Clostridium* spp. and an increase in the populations of *Bifidobacterium* spp. with intake of polyphenols (Fig. 9.4). This evidence suggests that the intake of green tea polyphenols may have an inhibitory effect on the growth of harmful *Clostridia* species. Colon cancer patients are often reported to have a high percentage composition of *Clostridia* and a low percentage of *Bifidobacteria*. Green tea polyphe-

nols, having the selective growth inhibitory effect on *Clostridia* and promoting effect on *Bifidobacteria*, may be helpful in regulating the balance of these bacteria in the intestine of colon cancer patients.

9.2.3 Prevention of foodborne bacterial infections

The delivery of food materials from production to consumer involves a series of steps in processing and storage. During these steps, the food may be exposed to different pathogenic bacterial contamination which may cause serious illness. The green tea polyphenols exhibited a strong antibacterial activity against several of those foodborne bacteria (Juneja et al., 2000; Sakanaka et al., 1997). Thermophilic spore-forming *Bacillus stearothermophilus*, a virulent bacterium resistant to high temperatures can spoil soft drinks stored in vending machines and Sakanaka et al. (2000) found green tea polyphenols to be very effective in inhibiting the growth of this bacterium. Similarly, the growth of several microbial and psychrophilic bacteria in ice-stored fish has been diminished with the application of polyphenols (Noriyuki et al., 2001). It has also been reported that polyphenols can inhibit the release of verotoxins from enterohemorrhagic *Escherichia coli* O157:H7 (Sugita-konishi et al., 1999). It was noticed that green tea polyphenols could inhibit *E. coli* infection in mice (Isogai et al., 1998) and the minimum inhibitory concentration for this organism was $<250\,\mu g\,ml^{-1}$ (Hara-kudo et al., 2001). A list of minimum inhibitory concentrations of green tea polyphenols on the growth of different foodborne bacteria of fish and animal origin is given in Table 9.7 (Sakanaka et al., 1997).

9.3 Antiviral activity

Besides their powerful antibacterial activity, green tea polyphenols have also exhibited strong activity against viral infections.

9.3.1 Prevention of viral infection

Nakayama et al. (1990) investigated the effect of polyphenols on the infectiousness of influenza A virus and influenza B virus in Madin-Darby canine kidney (MDCK). They observed a significant decrease in infection by the virus proportional to the concentration of green tea extract (Table 9.8). Nakayama et al. (1993) discovered that EGCg prevented the adsorption of the virus to MDCK. The catechin binds with hemagglutinin of influenza virus, inhibits adsorption to MDCK cells, and thus blocks infection.

The green tea polyphenols have also shown significant antiviral activity against rotavirus (Ebina, 1991; Hatta et al., 1989; Mukoyama et al., 1991), enterovirus (Mukoyama et al., 1991), *Vaccinia* virus, *Herpes simplex* virus, *Coxsackie* virus B6 and polio 1 virus (John and Mukundan, 1979). In all of

Table 9.7 Minimum inhibition concentration (MIC) of green tea extract on the growth of foodborne pathogens of fish and meat origin

Host and microorganisms	MIC ($\mu g ml^{-1}$)
Fish origin	
Ayu	
Vibrio anguillarum species	100–200
Vibrio spp.	200
Crawfish	
Vibrio spp.	50–400
Yellowfish	
Streptococcus spp.	700–900
Pasteurella piscicida spp.	100–200
Eel	
Edwardsiella tarda spp.	300–400
Vibrio spp.	200
Salmon	
Vibrio spp.	100–200
Meat origin	
Cattle	
Salmonella spp.	4000
Psedomonas spp.	500
Staphylococcus spp.	100–1000
Escherichia coli spp.	>4000
Pig	
Salmonella spp.	4000
Escherichia coli spp.	4000
Chicken	
Salmonella spp.	4000

Juneja et al., 2000

Table 9.8 Inhibition of influenza viruses A and B by green tea extract. Virus was mixed for 60 min with tea extract before adsorption to the MDCK cells. Inhibition of virus activity indicated by the reduction in plaque formation

Concentration of green tea extract ($\mu g ml^{-1}$)	Reduction in plaque formation (%)	
	Virus A	Virus B
0.05	78.5	59.4
0.1	87.0	80.0
0.2	98.8	94.0
0.4	100.0	98.6
0.8	100.0	100.0
1.0	100.0	100.0

Nakayama et al., 1990

these studies, EGCg was found to be an active component in the antiviral activity. The polyphenols, EGCg and ECg, were found to inhibit HIV (human immuno-deficiency virus), reverse transcriptase and cellular DNA and RNA polymerase (Nakane and Ono, 1990; Ono and Nakane, 1991). The minimum concentration of these components for 50% inhibition of HIV-reverse transcriptase was in the range of 0.01–0.02 $\mu g\,ml^{-1}$. It is likely that treatments for these viral infections could be developed from green tea polyphenols.

9.4 Antioxidant functions

It is well known that the free active radicals such as superoxide (O_2^-), hydroxyl radical (–OH) and radicals derived from hydrogen peroxide (H_2O_2) are very closely related to injury of cell membranes and DNA (Elias and Cohen, 1977). These free radicals were also found to be involved in ageing and in the initiation of several diseases. For example, diseases such as cancer, cardiovascular diseases and arthritis have a free radical component (Ames, 1983). There is no system to eliminate free radicals in our organs or tissues, physiological antioxidant protection is invoked as one of the major defense mechanisms in fighting free radical-induced, mediated and promoted disorders. This means that the most significant function of the antioxidants is believed to be disease prevention.

Vitamin C, vitamin E and β–carotene are well-known antioxidants. They appear to maintain the proper functioning of the immune system (Anon, 1990). Recently, the green tea polyphenols, particularly ECg and EGCg, were found to have the greatest activity of a number of antioxidants (Rice-Evans et al., 1996), when compared with other natural substances (Fig. 9.5). The efficacy of antioxidant activity of green tea polyphenols has been related to their chemical structures (Jovanovic et al., 1995; Rice-Evans et al., 1996; van Acker et al., 1996). Since antioxidant activity corresponds broadly with structures having the greatest number of hydroxyl groups, the green tea polyphenols (Fig. 9.1) with three –OH groups in the B ring (such as gallocatechins) and three –OH groups in the C ring (such as catechin gallates) have an advantage over others in scavenging the free radicals (Rice-Evans et al., 1996).

9.4.1 Prevention of cardiovascular diseases

A large percentage of human mortality occurs from cardiovascular disorders, which are mainly caused by atherosclerosis. Such cases are characterized by local thickening of the intema or innermost part of the arteries. A popular theory for the cause of atherosclerosis is that oxidation of low-density lipoprotein (LDL) leads to uptake via macrophage scavenger receptors in the arterial wall (Ross, 1993; Steinberg et al., 1989). It

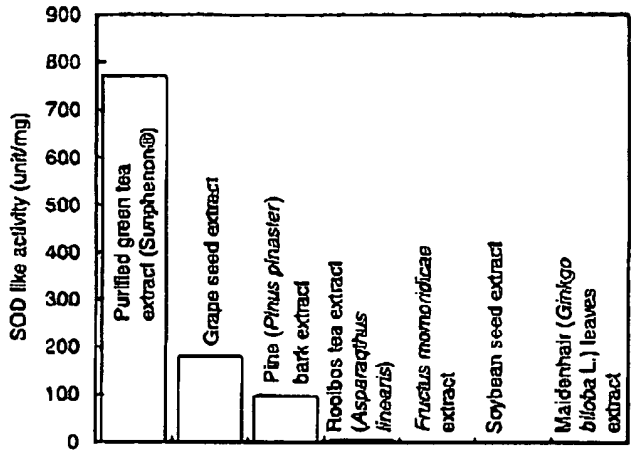

Fig. 9.5 Physiological antioxidant activity of various natural substances compared with purified green tea extract (Sunphenon®).

Table 9.9 Effect of green tea extract and its components on Cu-induced LDL peroxidation. LDL peroxidation is estimated fluorometrically as thiobarbituric acid (TBA)-reactive substances after 4 hours of incubation

Component concentration ($\mu g\,ml^{-1}$)	TBA-reactive substances ($nmol\,MDA\,ml^{-1}$) 0	0.1	0.5	2.5	25.0
Green tea extract	0.41	0.38	0.33	0.28	0.26
Polyphenols	0.42	0.29	0.25	0.23	0.22
Caffeine	0.42	0.40	0.40	0.38	0.37
Theanine	0.38	0.35	0.34	0.32	0.31

Yokozawa and Dong, 1997

therefore appears likely that substances capable of counteracting LDL oxidation would be of potential therapeutic interest. As antioxidants and scavengers of free radicals, green tea polyphenols were found to be a suitable natural substance to counter oxidation of LDL.

Yokozowa and Dong (1997) have tested the effect of green tea components *in vivo* on the oxidation of LDL which was time-dependently oxidized by copper (II) sulphate, leading to peroxidation, the extent of which was estimated fluorometrically as thiobarbituric acid (TBA)-reactive substances. The researchers observed a concentration-dependent decrease of LDL peroxidation with green tea components such as polyphenols and theanine (a major amino acid in green tea). However, caffeine did not influence peroxidation (Table 9.9). Miura et al. (1995) also found a similar effect of EOCg on copper-induced lipid peroxidation.

Table 9.10 Antioxidative activity of green tea polyphenols (final concentration, 1000 μM) compared with control and α-tocopherol and determined by the rabbit erythrocyte 'ghost' system

Component	Lipid peroxidation (%)
Control	100
α-tocopherol	24.1
C	29.8
EC	27.8
GC	36.3
EGC	27.8
ECg	17.1
EGCg	16.2

Ramarathnam et al., 1995

In another study, Ramarathnam et al. (1995) examined the effect of green tea polyphenols on lipid peroxidation in rabbit erythrocyte (red blood cell) membrane 'ghosts'. They observed substantial reduction of lipid peroxidation with different components of green tea polyphenols. Of the four catechins evaluated, ECg and EGCg produced strongest protection against lipid peroxidation, and are more active than the standard antioxidant α-tocopherol (Table 9.10).

Laboratory studies on animals suggest that green tea extracts suppress absorption of cholesterol from the digestive tract (Chisaka et al., 1988; Ikeda et al., 1992), and reduce the serum level of total cholesterol (Ali et al., 1990; Chisaka et al., 1988; Matsuda et al., 1986). A cross-sectional epidemiological study with Japanese men reported a significant inverse relationship between green tea consumption and serum levels of total cholesterol and triglycerides, as well as a positive association between green tea drinking and high density lipoprotein (HDL) cholesterol levels (Imai and Nakachi, 1995). These studies indicate the positive effects of green tea polyphenols on the management of cholesterol and indirectly on the prevention of cardiovascular diseases.

9.4.2 Prevention of cancer

The anticarcinogenic property of green tea polyphenols was first identified in the 1980s by Khan et al. (1988) and Wang et al. (1989a,b). Since then, extensive laboratory and epidemiological research has shown that green tea polyphenols can protect against a variety of cancer types (Table 9.11). Most of those studies were conducted either *in vitro* or *in vivo* using tissues or different organs or mice, respectively. Experimental evidence suggests that the polyphenolic antioxidants present in green tea inhibit cancer initiation

Table 9.11 List of publications referring to the anticarcinogenic properties of green tea polyphenols

Affected organ	Carcinogen	Reference
Skin	PAH, UVB	Katiyar et al. 1992, 1993a, 1999, 2000, 2001
	DMBA/TPA	Yang and Wang 1993;
	Benzoyl peroxide/TPA	Mukhtar et al. 1994; Huang et al. 1992
	4-NQO/TPA	Wang et al. 1989b, 1991, 1992a, 1994
Lung	B(a)P, NDEA, NNK	Wang et al. 1992b,c; Katiyar 1993b,c; Xu et al. 1992; Shi et al. 1994
Colon	Azoxymethane,	Yamane et al. 1991, 1995;
	DMH,	Inagake et al. 1995;
	MNNG	Narasiwa and Fukaura 1993
Prostate	Androgen, testosterone,	Gupta et al. 1999a,b, 2000; Liao and Hipakka 1995
Breast	-	Liao et al. 1995
Mammary gland	DMBA	Hirose et al. 1994
Esophagus	NMBA, Nitrososarcosine	Xu and Han 1990; Gao et al. 1990
Duodenum	ENNG	Fujita et al. 1989
Liver	Aflatoxin B_1, NDEA	Chen et al. 1987; Li 1991
Pancreas	N-nitroso-bis (2-oxypropyl)-amine	Harada et al. 1991

and its subsequent development. Much of the cancer chemopreventive effects of green tea have been attributed to the major polyphenolic constituent EGCg (Katiyar and Mukhtar, 1996). The polyphenols, in particular EGCg, seem to affect a variety of targets and pathways, and thus may be responsible for the exceptionally high cancer chemopreventive efficacy of these compounds. The targets and pathways modulated by polyphenols are:

1. MAPK, ERK2, JNK1.
2. Urokinase.
3. Apoptosis and cell cycle.
4. Protein tyrosine kinase (PTK) and ornithine decarboxylase (ODC) activities (Ahmad et al., 1997; Ahmad and Mukhtar, 1999).

Recently, Jankun et al. (1997) proved that the EGCg could effectively reduce the incidence of cancer and the size of tumors in humans. They identified that EGCg could fit well into the cavities formed by His 57, Asp 102,

Table 9.12 The intake of green tea extract or a single polyphenol 'EGCg' on urinary methylguanidine excretion during adenine-induced renal failure in rats

Substance concentration (mg day^{-1})	Methylguanidine in urine (μg ml^{-1}) 0	0.5	1.0	2.0
Green tea extract	71.9	58.0	62.6	44.7
Polyphenol EGCg	76.8	63.8	45.4	35.7

Yokozawa et al., 1992

Ser 195, Arg 35 and Arg A37 on the surface of urokinase and thereby suppress its carcinogenic enzyme activity.

9.4.3 Prevention of renal failure

Renal failure, a functional disorder of the kidney, occurs with the accumulation of highly oxidative uremic toxins; hence the symptoms of uremia, a high oxidative stress condition, were usually observed in renal failure patients (Fillit et al., 1981; Flament et al., 1986; Giardini et al., 1984; Kuroda et al., 1985). Among the various uremic toxins, methylguanidine was found to be the most pertinent in uremia. Methylguanidine is produced from creatinine by the hydroxyl radical (Jenaga et al., 1991; Nakamura et al., 1991; Yokozawa et al., 1991a,b and 1992). Green tea polyphenols known for their active free radical-scavenging activity inhibited the production of methylguanidine and thus alleviated renal failure both in animals (Sakanaka and Kim, 1997; Yokozawa et al., 1992, 1994, 1996a) and in humans (Sakanaka and Kim, 1997; Yokozawa et al., 1996b).

In rats, Yokozawa et al. (1992) examined the effect of green tea polyphenols on adenine-induced renal failure. They examined urinary methylguanidine excretion as an index of scavenging reaction. They administered rats with different doses of green tea polyphenols (as Sunphenon®) orally for 14 days after adenine administration for 20 days. A dose-dependent decrease in methylguanidine excretion was observed (Table 9.12), which was about 40% lower compared to control (administration of 2 mg green tea extract). A further decrease (about 50%) was noticed with the administration of exclusively EGCg at the rate of 0.5 mg day^{-1}. These results suggested that EGCg, the active component of green tea polyphenols, has very high scavenger activity against free radicals. In a clinical study, the effect of green tea polyphenols on the concentrations of serum creatinine and methylguanidine was observed in 50 dialysis patients (Yokozawa et al., 1996b). There was an 8% decrease in creatinine and 20% decrease in methylguanidine after 5 months' treatment (Fig. 9.6). These findings suggest

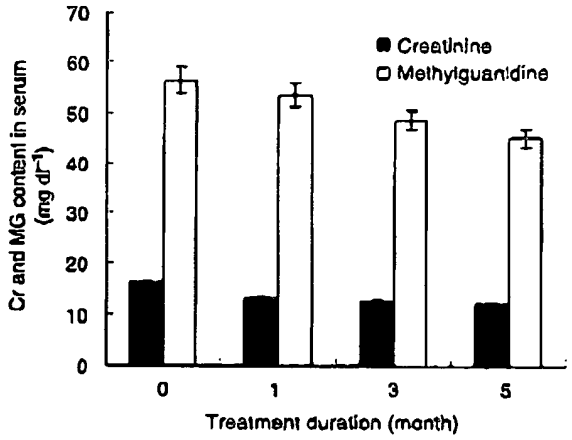

Fig. 9.6 Effect of green tea polyphenol on serum creatinine and methylguanidine contents in dialysis patients.

that green tea polyphenols have significant pharmacological effects in ameliorating the state of uremia in dialysis patients.

Green tea polyphenols have also relieved the pain and complications associated with renal failure. The post-dialysis pains in different parts of the body were relieved by the intake of green tea polyphenols (Yokozawa et al., 1996b). The application of polyphenols improved the glomerular function (Oura and Yokozawa, 1990; Yokozawa et al., 1993) and relieved renal hypertension (Yokozawa et al., 1994) by inhibiting mesangial cell proliferation and renal blood circulation, respectively. The 'antinephropathic' activity of green tea was confirmed by its powerful antioxidant activity against those free radicals that are involved in the complications of renal failure (Yokozawa et al., 1996a).

9.4.4 Prevention of allergy

Individuals are becoming increasingly susceptible to allergens – in particular to those in food and those that are airborne. Among the four types of classified allergies (Coombs and Gell, 1968), Type I allergy is most common in which people suffer from immediate hypersensitive reaction to allergens. The allergic reactions include a series of events; production of allergen specific IgE, its binding to Fc&RI receptor on the surface of mast cells or basophils, cross-linking of IgE by newly absorbed allergens, and release of chemical mediators such as histamine and leukotrienes (LT) from mast cells (Kawabe et al., 1987; Plaut and Zimmerman, 1993). Inhibition of any of these sequential steps may ease allergic symptoms. Compounds such as

Table 9.13 Effect of dietary green tea polyphenols (Sunphenon®) and fats on calcium ionophore A23187 induced histamine and leukotrienes (LT) released from rat peritoneal exudates cells (PEC)

Diet type	Chemical mediator release (ng 10^{-6} PEC cells)	
	Histamine	Leukotrienes
Without polyphenol		
Safflower oil	630	30.0
Perilla oil	609	13.0
Palm oil	769	27.0
With polyphenol		
Safflower oil	637	11.3
Perilla oil	595	8.7
Palm oil	821	20.0

Matsuo et al., 2000

antihistamine and LT synthesis inhibitors have shown antiallergic properties by inhibiting one of the above specific reactions.

Until recently there have been no specific studies that examined the exclusive effect of green tea polyphenols on allergy. However, studies have indicated that various polyphenols normally found in tea such as EGCg, ECg and EGC have inhibited LT release (Matsuo et al., 1996; Yamada et al., 1999) and histamine release (Maeda et al., 1989; Matsuo et al., 1997; Yamashita et al., 2000). These polyphenols were also found to inhibit allergies induced by Df-protease (Noguchi et al., 1999) and hyaluronidase (Maeda et al., 1990). Recently, Matsuo et al. (2000) have examined the combined effect of dietary green tea polyphenols and fats on the calcium ionophore A23187-induced release of chemical mediators (histamine and LT) in peritoneal exudates cells (PEC) of rats. They found that diets with a combination of green tea polyphenols have significantly inhibited LT release from PEC (Table 9.13). However, they did not find any effect on the release of histamine. These results suggest that the green tea polyphenols have a pharmacological effect in alleviating Type-I allergy by inhibiting LT production.

9.5 Conclusions

The secret behind the longevity and low incidence of certain diseases in the people of Japan might be linked to their consumption of green tea. Scientific evidence suggests that this simple beverage, rich in low molecular-weight polyphenols, protects the body from various ailments. Green tea

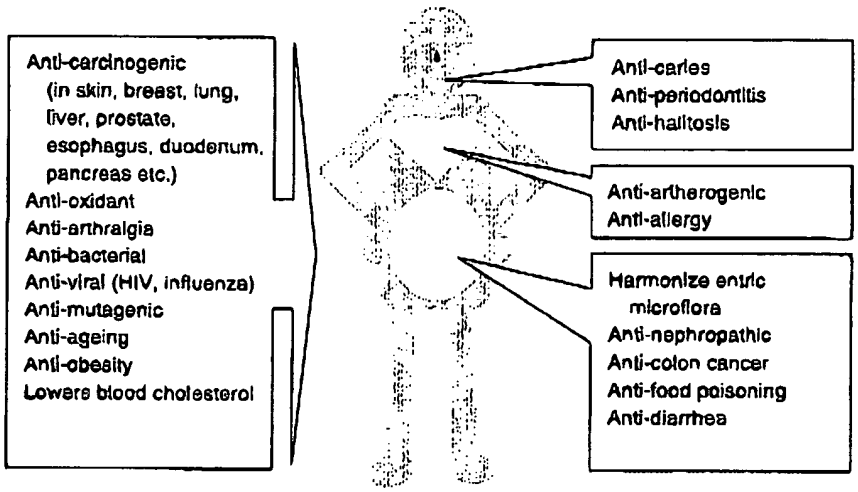

Fig. 9.7 Various pharmacological functions of green tea polyphenols in the human body.

polyphenols have powerful antioxidant and radical scavenger properties against disease-causing free radical species. In addition to this, green tea polyphenols also showed strong antimicrobial activity against various bacterial and viral infections.

Lately, several clinical studies showed various pharmacological functions of green tea polyphenols in humans (Fig. 9.7). These include 1) an improvement in oral health by suppression of those bacteria that cause dental caries, periodontal diseases and halitosis, 2) harmonization of enteric microflora by suppression of disease-induced bacteria, 3) prevention of foodborne bacterial and viral infections, 4) antiartherogenicity by regulating the oxidation of LDL, 5) potential anticarcinogenicity by suppression of various cancer-induced pathways, 6) antinephropathicity by easing the renal failure complications, and 7) antiallergicity by suppression of Type-1 allergic reactions.

The quest to find more pharmacological benefits of green tea extract has not yet ended. New findings suggest that the green tea polyphenols are effective in retarding cataracts (Thiagarajan et al., 2001), muscle necrosis (Buetler et al., 2002), obesity (Bell and Goodrick, 2002), liver damage (Arteel et al., 2002) and various cancer types (Yang et al., 2002). An increasing number of research findings are being published at approximately between 150 and 200 each year, indicating the vast physiological and pharmacological uses of green tea polyphenols. The trend in current research is largely focussed on the function of green tea polyphenols on lifestyle related disease, such as various types of cancer, cardiovascular disease, HIV, allergy and obesity and on ageing agents.

Green tea polyphenols are strong prophylactic and therapeutic agents, and could therefore offer the food industry an excellent opportunity for application as functional food ingredients in many processed foods. The green tea polyphenols, Sunphenon® and Sunkatol® from Taiyo Kagaku Co., Ltd., Japan, have been used widely in such products as chewing gum, candies, caramels, jelly beans and beverages in Japan and other countries. Several pharmacological, physiological functions and safety aspects of these polyphenols have been well tested around the world and have been reported in a number of articles and also published in a book, *Chemistry and Application of Green Tea* (CRC Press). In 1993, Sunphenon® was approved as a 'Food for Specific Health Use' (FOSHU) in Japan. The safe green tea polyphenols are a potential natural healthy ingredient for a good diet.

9.6 References

AHMAD N, FEYES D K, NIEMINEN A L, AGARWAL R and MUKHTAR H (1997), 'Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells', *J Natl Cancer Inst*, 89, 1881–6.

AHMAD N and MUKHTAR H (1999), 'Green tea polyphenols and cancer, Biologic mechanisms and practical implications', *Nutri Reviews*, 57, 78–83.

AHN Y J, KIM M, YAMAMOTO T, FUJISAWA T and MITSUOKA T (1990a), 'Selective growth responses of human intestinal bacteria to *Araliaceae* plant extracts', *Microb Ecol in Health and Disease*, 3, 223–9.

AHN Y J, SAKANAKA S, KIM M, KAWAMURA T, FUJISAWA T and MITSUOKA T (1990b), 'Effect of green tea extract on growth of intestinal bacteria', *Microb Ecol Health Disease*, 3, 335–8.

AHN Y J, KAWAMURA T, KIM M, YAMAMOTO T and MITSUOKA T (1991), 'The polyphenols, selective growth inhibitors of *Clostridium spp*', *Agric Biol Chem*, 55, 1425–6.

ALI M, AFZAL M, GUBLER C J and BURKA J F (1990), 'A potent thromboxane formation inhibitor in green tea leaves', *Prostaglandins Leukot Essent Fatty acids*, 40, 281–3.

AMES B N (1983), 'Dietary carcinogens and anticarcinogens, oxygen radical and regeneration diseases', *Science*, 221, 1256–64.

ANONYMOUS (1990), *Antioxidant nutrients and immune functions*, New York, Plenum Press, 262.

ANONYMOUS (1996), *Standard tables of food composition in Japan*, Tokyo, Resource Council of Science and Technology, 198–9.

ARTEEL G E, UESUGI T, BEVAN L N, GABELE E, WHEELER M D and MCKIM S E (2002), 'Green tea extract protects against early alcohol-induced liver injury in rats', *Biol Chem* 383, 663–70.

BELL S J and GOODRICK G K (2002), 'A functional food product for the management of weight', *Crit Rev Food Sci Nutr*, 42, 163–78.

BOKKENHEUSER V D (1983), 'Biotransformation of steroids', in Hentges D J (ed), *The human intestinal microflora in health and disease*, New York, Academic Press, 215–39.

BUETLER T M, RENARAD M, OFFORD E A, SCHNEIDER H and RUEGG U T (2002), 'Green tea extract decreases muscle necrosis in mdx mice and protects against reactive oxygen species', *Am J Clin Nutr*, 75, 749–53.

CHEN Z Y, YAN R Q and QIN G Z (1987), 'Effect of six edible plants on the development of aflatoxin B1-induced r-glutamyltranspepidase positive hepatocyte foci in rats', *Chinese J Cancer*, 9, 109–11.

CHISAKA T, MATSUDA H, KUBOMURA Y, MOCHIZUKI M, YAMAHARA J and FUJIMURA H (1988), 'The effect of crude drugs on experimental hypercholesterolemia, Mode of action (–)-epigallocatechin gallate in tea leaves', *Chem Pram Bull* 36, 227–33.

CHU D C and JUNEJA L R (1998), Chemical and physiological functions of green tea polyphenols, *Innov Food Tech Bull.*

COOMBS R R A and GELL P G H (1968), 'Classification of allergic reactions responsible for clinical hypersensitivity and disease', in Coombs R R A and Gell P G H (eds), *Clinical aspects of immunology*, Oxford, Blackwell Science, 575–96.

EBINA T (1991), 'Infantile gastroenteritis, prevention and treatment of rotavirus diarrhea', *Antibact Antifung Agents* 19, 349–58 (in Japanese).

ELIAS P S and COHEN A J (1977), *Radiation chemistry of major food components. Its relevance to the assessment of the wholesomeness of irradiated foods*, Amsterdam, Elsevier/North Holland Press.

FILLIT H, ELION E, SULLIVAN J, SHERMAN R and ZABRISKIE J B (1981), 'Thiobarbituric acid reactive material in uremic blood', *Nephron*, 29, 40–3.

FLAMENT J, GOLDMAN M, WATERLOT Y, DUPONT E, WYBRAN J and VANHERWEGEM J L (1986), 'Impairment of phagocyte oxidative metabolism in hemodialyzed patients with iron overload', *Clin Nephrol*, 25, 227–30.

FUJITA Y, YAMANE T, TANAKA M, KUATA K, OKUZUMI J, TAKAHASHI T, FUJIKI H and OKUDA T (1989), 'Inhibitory effect of (–)epigallocatechin gallate on carcinogensis with N-ethyl-N-nitro-N-nitrosoguandine in mouse duodenum', *Jpn J Cancer Res*, 80, 503–5.

GAO G D, ZHOU L F and QI C (1990), 'Initial study of antitumorigenesis of green tea: animal test and flow cytometry', *Tumor* 10, 42–4.

GARY L S and SHERWOOD L G (1984), 'Intestinal flora in health and disease', *Gastroenterology*, 86, 174–6.

GIARDINI O, GALLUCCI M T, LUDRANO R, TENORE G R, BANDINO D, SILVI I, RUBERTO U and CASCIANI C I (1984), 'Evidence of red blood cell membrane lipid peroxidation in haemodialysis patients', *Nephron*, 36, 235–7.

GOLDMAN P (1983), 'Biochemical pharmacology and toxicology involving the intestinal flora', in Hentges D J (ed), *The human intestinal microflora in health and disease*, New York, Academic Press, 241–63.

GUPTA S, AHMAD N and MUKHTAR H (1999a), 'Prostate cancer chemoprevention by green tea', *Seminars in Urologic Oncology*, 17, 70–6.

GUPTA S, AHMAD N, MOHAN R R, HUSAIN M M and MUKHTAR H (1999b), 'Prostate cancer chemoprevention by green tea: *in vitro* and *in vivo* inhibition of testosterone-mediated induction of ornithine decarboxylase', *Cancer Res*, 59, 2115–20.

GUPTA S, AHMAD N, NIEMINEN A L and MUKHTAR H (2000), 'Growth inhibition, cell-cycle dysregulation, and induction of apoptosis by green tea constituent (–)-epigallocatechin-3-gallate in androgen sensitive and adrogen-insensitive human prostate carcinoma cells', *Toxicol Appl Pharm*, 164, 82–90.

HAMADA S and SLADE H D (1980), 'Biology, immunology and cariogenicity of *Streptococcus mutans*', *Microbiol Rev*, 44, 331–84.

HARA-KUDO Y, OKUBO T, TANAKA S, CHU D C, JUNEJA L R, SAITO N and SUGITA-KONISHI Y (2001), 'Bacterial action of green tea extract and damage to the membrane of *Escherichia coli* O157,H7', *Biocont Sci*, 6, 57–61.

HARADA N, TAKABAYASHI F, OGUNI I and HARA Y (1991), 'Anti-promotion effect of green tea extracts on pancreatic cancer in golden hamster induced by N-nitroso-bis (2-oxo-propyl) amine', *Int Symp Tea Science*, Japan, 200–4.

HATTA H, SAKANAKA S, TSUDA N, KANATAKE H, YAMAMOTO T and EBINA T (1989), 'Anti-rotavirus agent in green tea', *37th Annual meeting of Japanese Society of Virologists*, Osaka. 327 (in Japanese).

HATTORI M, KUSUMOTO I T, NAMBA T, ISHIGAMI T and HARA Y (1990), 'Effect of tea polyphenols on glucan synthesis by glucosyltransferase from *Streptococcus mutans*', *Chem Pharm Bull*, 38, 717–20.

HENTGES D J (1983), 'Role of intestinal microflora in host defense against infection', in Hentges D J (ed), The *human intestinal microflora in health and disease*. New York, Academic Press, 311–31.

HIROSE M, HOSHIYA T, AKAGI K, FUTAKUCHI M and ITO N (1994), 'Inhibition of mammary gland carcinogensis by green tea catechins and other naturally occurring antioxidants in female Sprague-Dawley rats pretreated with 7,12-dimethylbenz(a) anthracene', *Cancer Lett*, 83, 149–56.

HU L (1986), *Traditional Chinese Medicine*, Beijing, Ancient Books Press, 1–2.

HUANG M T, HO C T, WANG Z Y, FERRARO T, FINNEGAN-OLIVE T, LOU Y R, MITCHELL J M, LASKIN J D, NEWMARK H, YANG C S and CONNEY A H (1992), 'Inhibitory effect of topical application of a green tea polyphenol fraction on tumor initiation and promotion in mouse skin', *Carcinogenesis*, 13, 947–54.

IENAGA K, NAKAMURA K, YAMAKAWA M, TOYOMAKI Y, MATUURA H, YOKOZAWA T, OURA H and NAKANO K (1991), 'The use of 13C-labelling to prove that creatinine is oxidized by mammal into creatol and 5-hydroxyl-methylhydantoin', *J Chem Soc Chem Commun*, 509.

IKEDA I, IMASATO Y, SASAKI E, NAKAYAMA M, NAGAO H, TAKEO T, YAYABE F and SUGANO M (1992), 'Tea catechins decrease micellar solubility and intestinal absorption of cholesterol in rats', *Biochim Biophys*, 1127, 141–6.

IMAI K and NAKACHI K (1995), 'Cross sectional study of effects of drinking green tea on cardiovascular diseases', *Br Med J*, 310, 693–6.

INAGAKE M, YAMANE T, KITAO Y, OYA K, MATSUMOTO H, NORIKAZU K, NAKATANI H, TAKAHASHI T, NISHIMURA H and IWASHIMA A (1995), 'Inhibition of 1,2-dimethylhydrazine-induced oxidative DNA damage by green tea extract in rat', *Jpn J Cancer Res*, 86, 1106–11.

ISHIHARA N, ARAKI T, TAMURA Y, INOUE M, NISHIMURA A, AOI N, CHU D C, JUNEJA L R and MORISHITA T (2001), 'Suppressive effects of green tea polyphenols on microbial growth and volatile basic nitrogen content in round form yellow fish (*Seriola quinqueradiata*) meat during ice storage', *Food Pres Sci*, 27, 269–75.

ISOGAI E, ISOGAI H, TAKESHI K and NISHIKAWA T (1998), 'Protective effect of Japanese green tea extract on gnotobiotic mice infected with an *Escherichia coli* O157,H7 strain', *Microbiol Immunol*, 42, 125–8.

JANKUN J, SELMAN S H and SWIERCZ R (1997), 'Why drinking green tea could prevent cancer', *Nature*, 387, 561.

JIN C, HAI L Q, ZHI P X, ZHONG L Z, TAO J Y, OGUNI I and HARA Y (1991), 'Anticaries activity of tea, epidemiology and the role of fluorine and catechins', *Uni Shizuoka Junior College Research Proceedings*, 4, 81–101.

JOHN J T and MUKUNDAN P (1979), 'Virus inhibition by tea, caffeine and tannic acid', *Indian J Med Res*, 69, 542–5.

JOVANOVIC S V, HARA Y, STEENKEN S and SIMIC M G (1995), 'Antioxidant potential of gallocatechins. A pulse radiolysis and laser photolysis study', *J Am Chem Sci*, 117, 9881–8.

JUNEJA L R, OKUBO T and HUNG P (2000), 'Catechins', in Naidu A S (ed), *Natural Food Antimicrobial Systems*, Washington D C, CRC Press, 381–98.

KAKUDA T, TAKIHARA T, SAKANE I and MORTELMANS K (1994), 'Antimicrobial activity of tea extracts against peridontopathic bacteria', *Nippon Nogeikagaku Kaishi*, 68, 241–3 (in Japanese).

KATIYAR S K, AGARWAL R, WOOD G S and MUKHTAR H (1992), 'Inhibition of 12-O-tetradecanoyl-phorbol-13-acetate-caused tumor promotion in 7,12-dimethylbenz(a)-anthracene-initiated SECAR mouse skin by a polyphenolic fraction isolated from green tea', *Cancer Res*, 52, 6890–7.

KATIYAR S K, AGARWAL R and MUKHTAR H (1993a), 'Protection against malignant conversion of chemically-induced benign skin papillomas to squamous cell carcinomas in SENCAR mice by a polyphenolic fraction isolated from green tea', *Cancer Res*, 53, 5409–12.

KATIYAR S K, AGARWAL R, ZAIM M T and MUKHTAR H (1993b), 'Protection against N-nitrosodiethylamine and benzo(a)pyrene-induced forestomach and lung tumorogenesis in A/J mice by green tea', *Carcinogenesis*, 14, 849–55.

KATIYAR S K, AGARWAL R and MUKHTAR H (1993c), 'Protective effects of green tea polyphenols administered by oral intubation against chemical carcinogen-induced forestomach and pulmonary neoplasia in A/J mice', *Cancer Lett*, 73, 167–72.

KATIYAR S K and MUKHTAR H (1996), 'Tea in chemoprevention of cancer, Epidemiologic and experimental studies', *Int J Oncol*, 8, 221–38.

KATIYAR S K, MATSUI M S, ELMETS C A and MUKHTAR H (1999), 'Polyphenolic antioxidant (–)-epigallocatechin-3-gallate from green tea reduces UVB-induced inflammatory responses and infiltration of leukocytes in human skin', *Photochem Photobiol*, 69, 148–53.

KATIYAR S K, PEREZ A and MUKHTAR H (2000), 'Green tea polyphenol treatment to human skin prevents formation of ultraviolet light B-induced pyrimidine dimmers in DNA', *Clin Can Res*, 6, 3864–9.

KATIYAR S K, AFAQ F, PEREZ A and MUKHTAR H (2001), 'Green tea polyphenol (–)-epigallocatechin-3-gallate treatment of human skin inhibits ultraviolet radiation-induced oxidative stress', *Carcinogenesis*, 22, 287–94.

KAWABE H, HAYASHI H and HAYASHI O (1987), 'Differential calcium effects on prostaglandin D2 generation and histamine release from isolated rat peritoneal mast cells', *Biochem Biophys Res Commun*, 143, 467–74.

KHAN W A, WANG Z Y, AKTHAR M, BICKERS D R and MUKHTAR H (1988), 'Inhibition of the skin tumorigenesity of (+/–)-7β8α-dihydroxyl-9 α,10 α-epoxy-7,8,9,10-tetra hydrobenzo(a)pyrene by tannic acid, green tea polyphenols and quercetin in Sencar mice', *Cancer Lett*, 42, 7–12.

KURODA M, ASAKA B, TOFUKU Y and TAKEDA R (1985), Serum antioxidant activity in uremic patients, *Nephron*, 41, 293–8.

LI Y (1991), 'Cooperative study on the inhibitory effect of green tea, coffee, and levamisole on the hepatocarcinogenic action of diethylnitrosamine', *Chinese J Cancer*, 13, 193–5.

LIAO S and HIPAKKA (1995), 'Selective inhibition of steroid 5 α-reductase (5AR) by tea epicatechin-3-gallate and epigallocatechin-3-gallate', *Biochem Biophy Res Commun*, 214, 833–8.

LIAO S, UMEKITA Y, GUO J, KOKONTIS O M and HIPAKKA R A (1995), 'Growth inhibition and regression of human prostate and breast cancer tumors in athymic mice by tea epigallocatechin gallate', *Cancer Len*, 96, 239–43.

LORSCHE W J (1986), 'Role of *Streptococcus mutans* in human dental decay', *Microbiol Rev*, 50, 353–80.

MAEDA Y, YAMAMOTO M, MASUI T, SUGIYAMA K, YOKOTA M, NAKAGOMI K, TANAKA H, TAKAHASHI I and KOBAYASHI T (1989), 'Inhibitory effect of tea extracts on histamine release from Mast cells (Studies on anti-allergic activity in Tea. I)', *Shokuhin Eiseishi*, 30, 295–9 (in Japanese).

MAEDA Y, YAMAMOTO M, MASUI T, SUGIYAMA K, YOKOTA M, NAKAGOMI K, TANAKA H, TAKAHASHI I, KOBAYASHI T and KOBAYASHI E (1990), 'Inhibitory effect of tea extracts on Hyaluronidase (Studies on anti-allergic activity in Tea. II)', *Shokuhin Eiseishi*, 31, 233–7 (in Japanese).

MARTIN R, LILLEY T H, BAILEY N A, FLASHAW C P, HASLAM E, MAGNOLATO D and BEGLEY N J (1986), 'Polyphenol-caffeine complexation', *J Chem Soc Chem Commun*, 105, 152–8.

MATSUDA H, CHISAKA T, KUBOMURA Y, YAMAHARA J, SAWADA T, FUJIMURA H and KIMURA H (1986), 'Effects of crude drugs on experimental hypercholestrolemia. I. Tea and its active principles', *J Ethnopharm*, 17, 213–24.

MATSUO N, YAMADA K, YAMASHITA K, SHOJI K, MORI M and SUGANO M (1996), 'Inhibitory effect of tea polyphenols on histamine and leukotriene B4 release from rat peritoneal cells', *In Vitro Cell Dev Biol*, 32, 340–4.

MATSUO N, YAMADA K, SHOJI K, MORI M and SUGANO M (1997), 'Effect of tea polyphenols on histamine release from rat basophilic leukemia (RBL-2H3) cells, the structure-inhibitory activity relationship', *Allergy*, 52, 58–64.

MATSUO N, YAMADA K, MORI M, SHOJI K, UEYAMA T, YUNOKI S, YAMASHITA K, OZEKI M and SUGANO M (2000), 'Inhibition by dietary tea polyphenols of chemical mediator release from rat peritoneal exudates cells', *Biosci Biotechnol Biochem*, 64, 1437–43.

MATSUZAKI T and HARA Y (1985), 'Antioxidant activity of tealeaf catechins', *Nippon Nogeikagaku kaishi*, 59, 129–34 (in Japanese).

MCCARTHY R E and SALYERS A A (1988), 'The effects of dietary fiber utilization on the colonic microflora', in Rowland I R (ed), *Role of the gut flora in toxicity and cancer*, New York, Academic Press, 295–313.

MITSUOKA Y (1984), *A Color atlas of anaerobic bacteria*, Tokyo, Shobunsha.

MIURA S, WATANABE J, SANO M, TOMITA T, OSAWA T, HARA Y and TOMITA I (1995), 'Effects of various natural antioxidants on the Cu^{2+}-mediated oxidative modification of low-density lipoprotein', *Biol Pharm Bull*, 18, 1–4.

MUKHTAR H, KATIYAR S K and AGARWAL R (1994), 'Green tea and skin-anticarcinogenic effects', *J Invest Dermatol*, 102, 3–7.

MUKOYAMA A, USHIJIMA H, NISHIMURA S, KOIKE H, TODA M, HABA Y and SHIMAMURA T (1991), 'Inhibition of rotavirus and enterovirus infections by tea extracts', *Jpn J Med Sci Biol*, 44, 181–6.

MURAYAMA N, SUZUKI T, SAKATA K, YAGI A and INA K (1991), 'NMR spectroscopic and computer graphics on the creaming down of tea', *Int symp tea science*, Shizuoka, Japan, Shizuoka, International Symposium of Tea Sciences, 145–9.

MUROI H and KUBO I (1993), 'Combination effects of antibacterial compounds in green tea against *Streptococcus mutans*', *J Agric Food Chem*, 41, 1102–5.

NAKAMURA K, INAGA K, YOKOZAWA M, FUJITUKA N and OURA H (1991), 'Production of methylguanidine from creatinine via creatol by active oxygen species, analysis of the catabolism *in vitro*', *Nephron*, 58, 42–5.

NAKANE H and ONO K (1990), 'Differential inhibitory effects of some catechin derivatives on the activities of Human Immunodeficiency Virus reverse transcriptase and cellular deoxyribonucleic and ribonucleic acid polymerases', *Biochem*, 29, 2841–5.

NAKAYAMA M, TODA M, OKUBO S and SHIMAMURA T (1990), 'Inhibition of influenza virus infection by tea', *Lett App Microbiol*, 11, 38–40.

NAKAYAMA M, SUZUKI K, TODA M, OKUBO S, HARA Y and SHIMAMURA T (1993), 'Inhibition of infectivity of influenza virus by tea polyphenols', *Antiviral Res*, 21, 289–99.

NARASIWA T and FUKAURA Y (1993), 'A very low dose of green tea polyphenols in drinking water prevents N-methyl-nitrosourea-induced colon carcinogenesis in F344 rats', *Jap J Cancer Res*, 84, 1007–9.

NAWASHIRO A, ASAKI N, NARA A, OGAWA T, KAMOI H, ITO A, KAMOI K and KAKUDA T (1996), 'Effects of periodontal pocket irrigation with a green tea extract on clinical signs and subgingival bacterial flora in periodontitis patients', *J Jap Soc Period*, 38, 97–106.

NOGUCHI Y, FUKUDA K, ATSUSHIMA A, HAISHI D, HIROTO M, KODERA Y, NISHIMURA H and INADA Y (1999), 'Inhibition of Df-protease associated with allergy disease by polyphenol', *J Agr Food Chem*, 47, 2969–72.

OIWA T, SAKANAKA S, KIM M, OZAKI T, KASHIWAGI M, HASEGAWA Y, YOSHIHARA Y and YOSHIDA S (1993), 'Inhibitory effect of human plaque formation by green tea polyphenols (Sunphenon)', *Jpn J Prd Dent*, 31, 247–50 (in Japanese).

OKUBO T, ISHIHARA N, OURA A, SERIT M, KIM M, YAMAMOTO T and MITSUOKA T (1992), '*In vivo* effects of tea polyphenol intake on human intestinal microflora and metabolism', *Biosci Biotech Biochem*, 56, 588–91.

OKUBO T and JUNEJA L R (1997), 'Effects of green tea polyphenols on human intestinal microflora', in Yamamoto T, Juneja L R, Chu D C and Kim M (eds), *Chemistry and applications of green tea*, New York, CRC Press, 109–21.

ONISI M (1985), 'The feasibility of a tea drinking program for dental public health in primary schools', *J Dent Hlth*, 35, 134–44.

ONISI M, SHIMURA N, NAKAMURA C and SATO M (1981a), 'A field test on the caries prevention effect of tea drinking', *J Dent Hlth*, 31, 13–19.

ONISI M, OZAKI F, YOSHINO F and MURAKAMI Y (1981b), 'An experimental evidence of caries preventive activity of non-fluoride component in tea', *J Dent Hlth*, 31, 86–90.

ONO K and NAKANE H (1991), 'Catechins as a novel class of inhibitors for HIV-reverse transcriptase and DNA polymerases', *Int symp tea science*, Japan, 277–81.

OTAKE S, MAKIMURA M, KUROKI T, NISHIHARA Y and HIRASAWA M (1991), 'Anticaries effect of polyphenolic compounds from Japanese green tea', *Caries Res*, 25, 438–43.

OURA H and YOKOZAWA T (1990), 'Pharmacological action of tannins', *J Trad Sino-Jpn Med*, 11, 67–74.

PLAUT M and ZIMMERMAN E M (1993), 'Allergy and mechanisms of hypersensitivity', in Paul W E (ed), *Fundamental immunology*, New York, Raven press, 1399–425.

RAMARATHNAM N, OSAWA T, OCHI H and KAWAKISHI S (1995), 'The contribution of plant food antioxidants to human health', *Trends Food Sci Tech*, 6, 75–82.

RICE-EVANS C A, MILLET N J and PAGANGA G (1996), 'Structure-antioxidant activity relationships of flavnoids and phenolic acids', *Free Radical Biol Med*, 20, 933–56.

RICHARD F C, GOUPY P M, NICOLAS J J, LCOMBE J M and PAVIRA A A (1991), 'Cysteine as an inhibitor of enzymatic browning. 1. Isolation and characterization of addition compounds formed during oxidation of phenols by apple polyphenolic oxidase', *J Agric Food Chem*, 39, 841–5.

ROSS R (1993), 'The pathogenesis of atherosclerosis, a perspective for the 1990s', *Nature*, 362, 801–9.

ROWLAND I R, MALLETT A K and WISE A (1985), 'The effect of diet on the mammalian gut flora and its metabolic activities', *CRC Critical Reviews in Toxicology*, New York, CRC Press, 31.

SAKANAKA S (1997), 'Green tea polyphenols for prevention of dental caries', in Yamamoto T, Juneja L R, Chu D C and Kim M (eds), *Chemistry and applications of green tea*, New York, CRC Press, 87–101.

SAKANAKA S, KIM M, TANIGUCHI M and YAMAMOTO T (1989), 'Antibacterial substance in Japanese green tea extract against *Streptococcus mutants*, a cariogenic bacterium', *Agric Biol Chem*, 53, 2307–11.

SAKANAKA S, SATO T, KIM M and YAMAMOTO T (1990), 'Effects of green tea polyphenols on glucan synthesis and cellular adherence of cariogenic *streptococci*', *Agric Biol Chem*, 54, 2925–7.

SAKANAKA S, SHIMURA N, AIZAWA M, KIM M and YAMAMOTO T (1992), 'Preventive effect of green tea polyphenols against dental caries in conventional rats', *Biosci Biotech Biochem*, 56, 592–4.

SAKANAKA S, AIZAWA M, KIM M and YAMAMOTO T (1996), 'Inhibitory effects of green tea polyphenols on growth and cellular adherence of an oral bacterium, *Porphyromonas gingivalis*', *Biosci Biotech Biochem*, 60, 745–9.

SAKANAKA S and KIM M (1997), 'Suppressive effect of uremic toxin formation by tea polyphenols', in Yamamoto T, Juneja L R, Chu D C and Kim M (ed), *Chemistry and applications of green tea*, New York, CRC Press, 75–86.

SAKANAKA S, OKUBO Y, AKACHI S, MABE K and MATSUMOTO M (1997), 'Tables of data on the antimicrobial activities of green tea extracts', in Yamamoto T, Juneja L R, Chu D C and Kim M (eds), *Chemistry and applications of green tea*, New York, CRC Press, 145–9.

SAKANAKA S, JUNEJA L R and TANIGUCHI M (2000), 'Anitimicrobial effects of green tea polyphenols on termophilic spore-forming bacteria', *J Biosci Bioeng*, 90, 81–5.

SALYERS A A and LEEDLE J A Z (1983), 'Carbohydrate metabolism in the human colon', in Hentges D J (ed), *The human intestinal microflora in health and disease*, New York, Academic Press, 129–46.

SEKIYA J, KAJIWARA T, MONMA T and HATANAKA A (1984), 'Interaction of tea catechins with proteins, Formation of protein precipitate', *Agric Biol Chem*, 48, 105–8.

SHI S T, WANG Z Y, THERESA J S, HONG J, CHEN W, HO C and YANG C S (1994), 'Effects of green tea and black tea on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone bioactivation, DNA methylation and lung tumorigenesis in A/J Mice', *Cancer Res*, 54, 4641–7.

STEINBERG D, PARTHSARATHY S, CAREW T E (1989), 'Modifications of low-density lipoprotein that increase its atherogenicity', *N Engl J Med*, 320, 915–24.

SUGITA-KONISHI Y, HARA-KUDO Y, AMANO F, OKUBO T, AOI N, IWAKI M and KUMAGAI S (1999), 'Epigallocatechin gallate and gallocatechin gallate in green tea catechins inhibit extracellular release of vero toxin from enterohemorrhagic *Escherichia coli* O157:H7', *Biochem Biophy Acta*, 1472, 42–50.

TERAJIMA T, TERAJIMA H, TOGASHI K, HASEGAWA Y, TAKAHASHI R, OZAKI T, SAKANAKA S, KIM M and YOSHIDA S (1997), 'Preventive effects of tea polyphenols (Sunphenon™) on plaque formation in men', *Nihon Univ Dent J*, 7, 654–9 (in Japanese).

THIAGARAJAN G, CHANDANI S, SUNDARI C S, RAO S H, KULKARNI A V and BALASUBRAMANIAN D (2001), 'Antioxidant properties of green and black tea and their potential ability to retard the progression of eye lens cataract', *Exp Eye Res*, 73, 393–401.

TOKITA F, ISHIKAWA M, SHIBUYA K, KOSHIMIZU M and ABE R (1984), 'Deodorizing activity of some plant extracts against methyl mercaptan', *Nippon Nogeikagaku Kaishi*, 58, 585–9 (in Japanese).

TSUCHIYA H, SATO M, KATO H, OKUBO T, JUNEJA L R and KIM M (1997), 'Simultaneous determination of catechins in human saliva by high performance liquid chromatography', *J Chromatography*, 703, 253–8.

UI M, YASUDA H, SHIBATA M, MARUYAMA T, HOTTA H, HARA T and YASUDA T (1991), 'Aantioxidant activity of tea leaf catechins', *Nippon Shokuhin Kogyo Gakkaishi*, 38, 1098–102 (in Japanese).

VAN ACKER S A B E, VAN DEN BERG D J, TROMP M N J L, GRIFFIOEN D H, VAN BENNEKOM W P, VAN DER VIJGH W J F and BAST A (1996), 'Structural aspects of antioxidant activity of flavonoids', *Free Radical Biol Med*, 20, 331–42.

WANG Z Y, CHENG S J, ZHOU Z C, ATHAR M, KHAN W A, BICKERS D R and MUKHTAR H (1989a), 'Antimutagenic activity of green tea polyphenols', *Muta Res*, 223, 273–85.

WANG Z Y, KHAN W A, BICKERS D R and MUKHTAR H (1989b), 'Protection against polycyclic aromatic hydrocarbon-induced skin tumor initiation in mice by green tea polyphenols', *Carcinogenesis*, 10, 411–15.

WANG Z Y, AGARWAL R, BICKERS D R and MUKHTAR H (1991), 'Protection against ultraviolet B radiation-induced photo-carcinogenesis in hairless mice by green tea polyphenols', *Carcinogenesis*, 12, 1527–30.

WANG Z Y, HUANG M-T, FERRARO T, WONG C-Q, LOU Y-R, IATROPOPULOS M, YANG C S and CONNEY A H (1992a), 'Inhibitory effect of green tea in the drinking water on tumerogenesis by ultraviolet light and 12-O-tetradecanoylphorbol-13-acetate in the skin of AKH-1 mice', *Cancer Res*, 52, 1162–70.

WANG Z Y, AGARWAL R, KHAN W A and MUKHTAR H (1992b), 'Protection against benzo(a)pyrene and N-nitrosodiethyl-amine-induced lung and forestomach tumoroginesis in A/J mice by water extracts of green tea and lico-rice', *Carcinogenesis*, 13, 1491–4.

WANG Z Y, HONG J Y, HAUNO M T, REUHL K R, CONNEY A H and YANG C S (1992c), 'Inhibition of N-nitrosodiethylamine and 4-(methylnitrosomaino)-1-(3-pyridyl)-1-butanone-induced tumorigensis in A/J mice by green tea and black tea', *Cancer Res*, 52, 1943–7.

WANG Z Y, HUANG M-T, LOU Y-R, XIE J-G, REUHL K R, NEWMARK H L, HO C-T, YANG C S and CONNEY A H (1994), 'Inhibitory effects of black tea, green tea, decaffeinated black tea and decaffeinated green tea on ultraviolet B light-induced skin carcinogenesis in 7,12-dimethylbenz(a)anthracene-initiated SKH-1 mice', *Cancer Res*, 54, 3428–35.

XU Y and HAN C (1990), 'The effect of Chinese tea on the occurrence of esophageal tumors induced by N-nitosome-methylbenzylamine formed *in vivo*', *Biomed Environ Sci*, 3, 406–12.

XU Y, HO C T, AMIN S G, HAN C and CHUNG F L (1992), 'Inhibition of tobacco-specific nitrosamine-induced lung tumorigenesis in A/J mice by green tea and its major polyphenol as antioxidants', *Cancer Res*, 52, 3875–9.

YAMADA K, SHOJI K, MORI M, UEYAMA T, ATSUO N, OKA S, NISHIYAMA K and SUGANO M (1999), 'Structure-activity relationship of polyphenols on inhibition of chemical mediator release from rat peritoneal exudates cells', *Vitro Cell Dev Biol Animal*, 35, 169–74.

YAMANE T, HAGIWARA N, TATEISHI M, AKCHI S, KIM M, OKUZUMI J, KITAO Y, INAGAKE M, KUWATA K and TAKAHASHI T (1991), 'Inhibition of azoxymethane-induced colon carcinogenesis in rat by green tea polyphenol fraction', *Jpn J Cancer Res*, 82, 1336–40.

YAMANE T, TAKAHASHI T, KUWATA K, OYA K, INAGAKE M, KITAO Y, SUGANUMA and FUJIKI H (1995), 'Inhibition of N-methyl-N'-nitro-N-nitrosoguanidine-induced carcinogensis by (−)-epigallocatechin gallate in the rat glandular stomach', *Cancer Res*, 55, 2081–4.

YAMASHITA K, UZUKI Y, MATSUI T, YOSHIMARU T, YAMAKI M, KARASAKI M S, HAYKAWA S and SHIMIZU K (2000), 'Epigallocatechin gallate inhibits histamine release from rat basophilic leukemia (RBL-2H3) cells, role of tyrosine phosphorylation pathway', *Biochem Biophys Res Commun*, 274, 603–8.

YANG C S and WANG Z Y (1993), 'Tea and cancer', *J Natl Cancer Inst*, 85, 1038–49.

YANG C S, MALIAKAL P and MENG X (2002), 'Inhibition of carcinogensis by tea', *Ann Rev Pharmacol Toxicol*, 42, 25–54.

YASUDA H and UI M (1992), 'Deodorant effect of plant extracts of the family Rosaceae against methyl mercaptan', *Nippon Nogeikagaku Kaishi*, 66, 1475–9 (in Japanese).

YASUDA H and ARAKAWA T (1995), 'Deodorizing mechanism of (−)-epigallocatechin gallate against methyl mercaptan', *Biosci Biotech Biochem*, 59, 1232–6.

YOKOZAWA T, FUJITUKA N, OURA H, IENAGA K and NAKAMURA K (1991a), 'Comparison of methylguanidine production from creatinine and creatol *in vivo*', *Nephron*, 58, 125–9.

YOKOZAWA T, FUJITUKA N and OURA H (1991b), 'Contribution of hydroxyl radical to the production of methylguanidine from creatinine', *Nephron*, 59, 662–5.

YOKOZAWA T, OURA H, SAKANAKA S and KIM M (1992), 'Effect of tannins in green tea on the urinary methylguanidine excretin in rats indicating a possible radical scavenging action', *Biosci Biotech Biochem*, 56, 896–9.

YOKOZAWA T, OURA H, HATTORI M, IWANO M, DOHI K, SAKANAKA S and KIM M (1993), 'Inhibitory effect of tannin in green tea on the proliferation of mesangial cells', *Nephron*, 65, 596–600.

YOKOZAWA T, OURA H, SAKANAKA S, ISHIGAKI S and KIM M (1994), 'Depressor effect of tannin in green tea on rats with renal hypertension', *Biosci Biotech Biochem*, 58, 855–8.

YOKOZAWA T, CHUNG H Y, HE L Q and OURA H (1996a), 'Effectiveness of green tea tannin on rats with chronic renal failure', *Biosci Biotech Biochem*, 60, 1000–5.

YOKOZAWA T, OURA H, SHOBATA T, ISHIDA K, KANEKO M, HASEGAWA M, SAKANAKA S and KIM M (1996b), 'Effects of green tea tannin in dialysis patients', *J Trad Med*, 13, 124–31.

YOKOZAWA T and DONG E (1997), 'Influence of green tea and its three major components upon low density lipoprotein oxidation', *Exp Toxic Pathol* 49, 329–35.

YU H, OHO T, TAGOMORI S and MORIOKA T (1992), 'Anticariogenic effects of green tea', *Fukuoka Acta Med*, 83, 174–80.